European Journal of Phycology

ISSN: 0967-0262 (Print) 1469-4433 (Online) Journal homepage: https://www.tandfonline.com/loi/tejp20

Cryptic, alien and lost species: molecular diversity of *Ulva sensu lato* along the German coasts of the North and Baltic Seas

S. Steinhagen, R. Karez & F. Weinberger

To cite this article: S. Steinhagen, R. Karez & F. Weinberger (2019) Cryptic, alien and lost species: molecular diversity of *Ulva sensu lato* along the German coasts of the North and Baltic Seas, European Journal of Phycology, 54:3, 466-483, DOI: 10.1080/09670262.2019.1597925

To link to this article: https://doi.org/10.1080/09670262.2019.1597925

EUROPEAN JOURNAL OF PHYCOLOGY
2019, VOL. 54, NO. 3, 466–483
https://doi.org/10.1080/09670262.2019.1597925

Cryptic, alien and lost species: molecular diversity of *Ulva sensu lato* along the German coasts of the North and Baltic Seas

S. Steinhagen[a], R. Karez[b] and F. Weinberger[a]

[a]GEOMAR Helmholtz Centre for Ocean Research Kiel, Marine Ecology Department, Düsternbrooker Weg 20, 24105 Kiel, Germany; [b]State Agency for Agriculture, Environment and Rural Areas, Schleswig-Holstein, Hamburger Chaussee 25, 24220 Flintbek, Germany

ABSTRACT

DNA barcoding analysis, using *tuf*A, revealed considerable differences between the expected and observed species inventory of *Ulva sensu lato* in the Baltic and North Sea areas of the German state of Schleswig-Holstein. Of 20 observed genetic entities, at least four (*U. australis, U. californica, U. gigantea* and *Umbraulva dangeardii*) had been introduced recently, whereas three others (one *Ulva* sp. and two *Blidingia* spp.) could not be identified at the species level and could also represent recently introduced species. In addition, the observed distributions of *Kornmannia leptoderma* and *U. rigida* were much more extensive than indicated by historical records, whereas *Blidingia minima* and *Gayralia oxysperma* were absent or much less common than expected. Barcoding analysis also revealed that both *U. tenera* (type material) and *U. pseudocurvata* (historical vouchers) from Helgoland, an off-shore island in the North Sea, actually belong to *U. lactuca*, a species that appears to be restricted to this island. Furthermore, past morphological descriptions of *U. intestinalis* and *U. compressa* have apparently been too restrictive and have been responsible for numerous misidentifications. The same is true for *U. linza*, which, in northern Germany, clusters into two genetically closely related but morphologically indistinguishable entities. One of these entities is present on Helgoland, while the second is present on North Sea and Baltic Sea mainland coasts.

ARTICLE HISTORY Received 26 October 2018; revised 28 January 2019; accepted 2 February 2019

KEYWORDS *Blidingia*; *Gayralia*; *Kornmannia*; *Monostroma*; *Protomonostroma*; *tufA*; *Ulva*; *Umbraulva*

Introduction

Macroalgae of the orders Ulvales and Ulotrichales are ubiquitous inhabitants of fully marine and brackish coastal waters. Several macroalgal species have recently increased, owing to opportunistic lifestyles and the capacity to benefit from eutrophication and other anthropogenic impacts, and the ability to accurately identify such taxa has become much more important (Charlier *et al.*, 2007, 2008; Smetacek & Zingone, 2013). However, the identification of certain macroalgae, such as the genus *Ulva*, is notoriously difficult (Koeman & Van den Hoek, 1981, 1982*a*, 1982*b*, 1984; Hoeksema & Van den Hoek, 1983; Brodie *et al.*, 2007), with the morphological instability of specific *Ulva* species being attributed to variation in salinity (Reed & Russell, 1978; Steinhagen *et al.*, 2018*b*), nutrient concentrations (Blomster *et al.*, 2002; Steinhagen *et al.*, 2018*b*) and bacterial associations (Spoerner *et al.*, 2012; Wichard, 2015), as well as to an elevated tendency for mutagenesis (Wichard, 2015). As a consequence, morphological plasticity (i.e. multiple morphotypes within species) or cryptic speciation may hinder identification and lead to taxonomic confusion. Such identification problems have been confirmed by DNA barcoding studies (e.g. Blomster *et al.*, 1998, 2002; Tan *et al.*, 1999; Hayden & Waaland, 2002; Hayden *et al.*, 2003; Shimada *et al.*, 2003; Brodie *et al.*, 2007; Heesch *et al.*, 2009; Wolf *et al.*, 2012; Kirkendale *et al.*, 2013), which have reported that the historical separation of *Enteromorpha* (for tubular 'species') and *Ulva* (for sheet-like taxa) is artificial and does not reflect phylogenetic relationships, as predicted by Linnaeus (1753; Hayden *et al.*, 2003). The genera *Enteromorpha* and *Ulva* were consequently synonymized and the currently accepted genus *Ulva* includes tubular, sheet-like and mixed-morphology taxa. Thus, allegedly unique morphological characteristics that were indicated in past species descriptions, and subsequently used in identification keys, are often uninformative, whereas molecular methods allow for reliable species differentiation (Blomster *et al.*, 1998, 2002; Hayden *et al.*, 2003; Brodie *et al.*, 2007). In particular, *tuf*A has been reported as a useful marker for identifying green algae (Saunders & Kucera, 2010). However, DNA-based species identification remains ambiguous when reference sequences of type material are missing, as is the case for most of the Ulvales and Ulotrichales. The DNA quality of historical voucher specimens is often low, thereby hampering sequencing efforts (Staats *et al.*, 2011). Therefore, both molecular and morphological methods are still needed to link taxonomic concepts that were originally based on morphology with molecular traits (Hillis, 1987).

CONTACT S. Steinhagen ssteinhagen@geomar.de

Published online 14 Jun 2019

The documentation of seaweeds in northern Germany has been conducted since the mid-19th century, and seaweeds of the small island of Helgoland, located in the SE North Sea, have received much attention from marine botanists and phycologists, making Helgoland among the best-studied seaweed habitats in Europe (Bartsch & Kuhlenkamp, 2000). The solid rock pedestal of Helgoland provides a natural substratum for a macrophytobenthos in a fully marine environment and comprises a unique habitat in Germany (Reinke, 1889; Bartsch & Kuhlenkamp, 2000). However, even the most recent comprehensive descriptions of Helgoland's macroalgae (Kornmann & Sahling, 1977, 1983, 1994) were exclusively based on morphological identification. Bartsch & Kuhlenkamp (2000) also included rare and doubtful species and summarized taxonomic changes. Furthermore, the understanding of macroalgal species diversity on Helgoland is only transferable to a limited extent to Germany's mainland coasts, which differ extensively in ecological conditions.

The tidal Wadden Sea is another fully marine ecosystem (salinity 30–33) within the North Sea, but it mainly consists of extended sand and mud flats, with relatively little hard substrate, and the German coast of the Baltic Sea is brackish, lacks tides, and is mainly composed of stones, gravel and sand (Rönnbäck *et al.*, 2007). Furthermore, except for general identification keys (Rothmaler, 1984; Pankow, 1990), information about the identity and abundance of macroalgae in the Wadden and Baltic Sea areas of Germany is relatively sparse. Based on a summary of literature records, Schories *et al.* (2009) described the distribution of macroalgae along the coast of Germany. However, the taxonomic concepts underlying historical records are often unclear and records based on molecular species identification are still sparse for the area.

Accordingly, the aim of the present study was to reassess the diversity of *Ulva sensu lato* at geographically separated areas along the coasts of the Baltic and North Seas in the German state of Schleswig-Holstein, as well as on Helgoland. The survey included both DNA barcoding and classical morphological identification approaches, and both field-collected and herbarium specimens were examined which allowed for the detection of several cryptic or newly introduced species and for the identification of several historical misinterpretations.

Materials and methods

Sample collection

Samples of *Ulva sensu lato* were collected from 127 sites throughout the state of Schleswig-Holstein, Germany (Fig. 1), including sites in the Wadden Sea (n = 44), Baltic Sea (n = 73) and on Helgoland (n = 10). The sites represented a variety of habitats, such as estuaries, overflow basins and drainage channels, within each of the ecosystems. The sites were spread over 536 km along the coast of the Baltic Sea and over 466 km along the coast of the North Sea, with a maximum distance between sites of less than 25 km. Full data on the collection sites are available in Supplementary table S1. To ensure that seasonal species were sampled, collections were conducted during both summer (July–August 2014 and August–September 2015) and spring (April 2015 and March 2016). Single locations were also visited in 2017 and 2018, and only a limited number of sites were visited during winter (November 2014–early March 2015) owing to lower green algal growth. Sites along the coast of the North Sea (mainly groynes, bulwarks, rocks and mudflats) were sampled during low tide, whereas sites along the coast of the Baltic Sea were sampled when water levels were low using waders and an aquascope, which allowed for sampling to a depth of 1.2–1.5 m below mean sea level. Additional sampling (n = 3) was undertaken by divers in August 2014. Representative specimens were collected for each morphotype that was observed at each sample site, and epiphytes were also collected from host specimens. The collected thalli were stored in a cool box (~10°C) and transported to the laboratory.

Morphological analysis

Pre-identification was based on typical morphological characters (e.g. overall thallus morphology, cell form, cell arrangement, number of pyrenoids per cell, etc.) using identification keys (Koeman & Van den Hoek, 1981, 1982*a*, 1982*b*, 1984; Hoeksema & Van den Hoek, 1983), and morphological characters were recorded separately at basal, middle and apical-thallus parts using light microscopy. Lugol's solution (iodine-potassium iodide) was used to stain starch-containing compartments, such as pyrenoids. After morphological analysis, epiphyte-free pieces of remaining thallus tissue (1 cm^2) or complete smaller thalli were either frozen and lyophilized or dried in silica gel for future molecular analysis.

Molecular analysis

Total DNA was extracted from lyophilized or silica-dried samples using the Invisorb Spin Plant Mini Kit (Stratec, Birkenfeld, Germany), according to the manufacturer's protocol, and the plastid-encoded DNA barcoding marker *tuf*A was PCR amplified using the primers tufGF4 (Saunders & Kucera, 2010) and tufAR (Famà *et al.*, 2002). The following conditions for amplification were used: initial denaturation at 94°C for 4 min; 38 cycles of 94°C for 1 min, 55°C for 30 s and 72°C for 1 min; then a final extension of 72°C for 7 min. Both strands of the

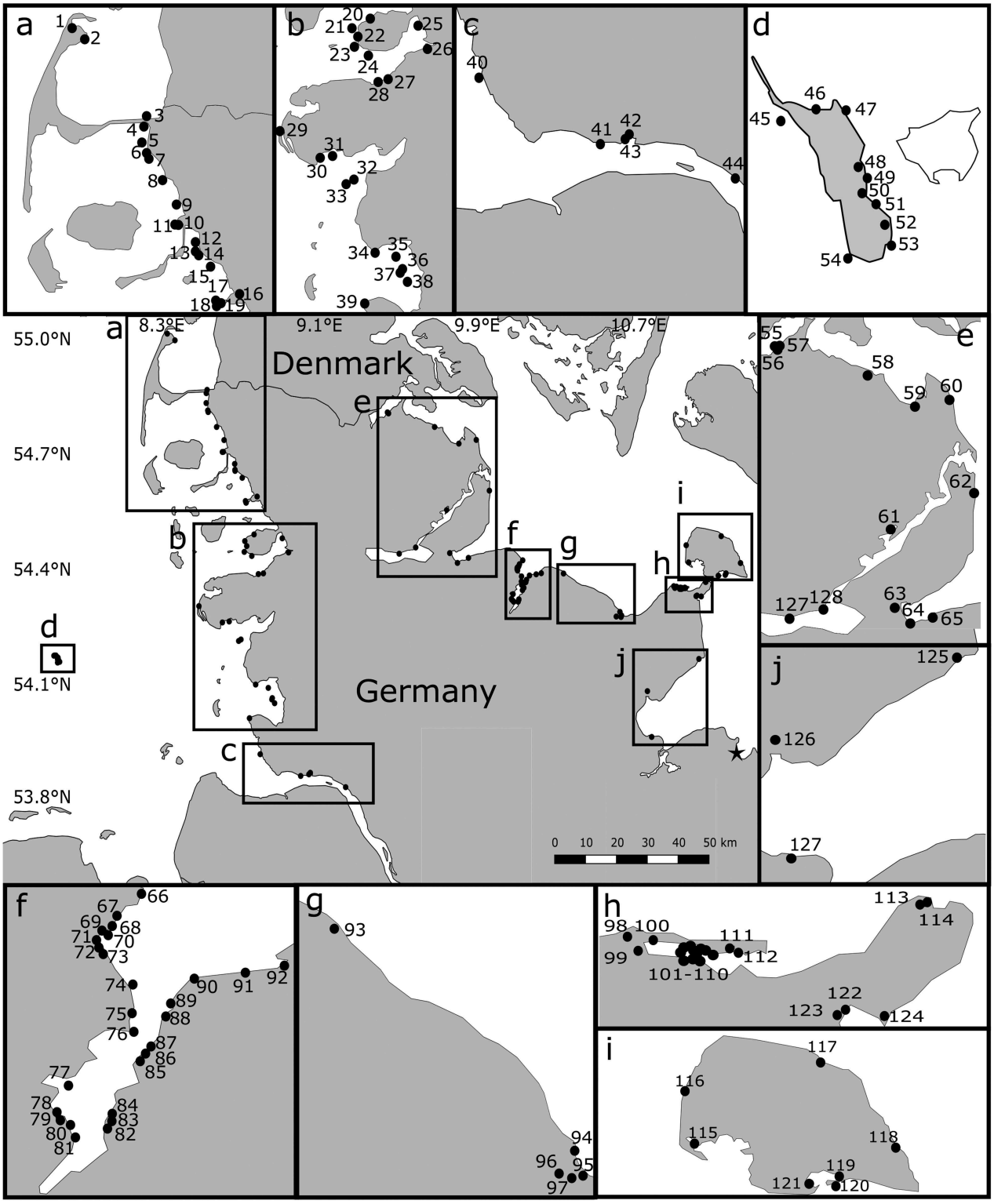


Fig. 1. Map of sampling sites in northern Germany. Insets a–j provide higher resolution. Numbers 1–126 cross-reference to Table 2 and Supplementary table S1, whereas numbers 127 and 128 indicate the sampling sites at Winning and Brodersby. The asterisk indicates a previously investigated site in Wohlenberg.

purified amplicons were directly sequenced by GATC Biotech (Konstanz, Germany) and both sequence alignment and reciprocal editing were performed using Sequencher (v. 4.1.4; Gene Codes Co., Ann Arbor, Michigan). The resulting sequences were uploaded to GenBank (Supplementary table 1). Sequence alignment was performed using MAFFT (Katoh *et al.*, 2002), whereas editing was done visually with Sequencher (v. 4.1.4, Gene Codes Corporation, Ann Arbor, Michigan). The alignment represented a 777 bp portion of the *tuf*A gene. An optimal substitution model was determined using MrModeltest software version v. 2.2. (Nylander, 2004) and found to be GTR+G+I. Subsequently, maximum likelihood analysis was performed using RAxML (v. 8; Stamatakis, 2014) with 1000 bootstrap

iterations and the suggested substitution model, and Bayesian inference was performed using MrBayes (v. 3.2.2; Ronquist *et al.*, 2012) with four simultaneously running Markov Chain Monte Carlo chains for 5×10^6 generations. The run was ended automatically when the standard deviation of split frequencies dropped below 0.01. Reference sequences from GenBank were also included in the analyses, with preference given to annotated sequences published in peer-reviewed articles. The trees were rooted by an outgroup that contained *Urospora penicilliformis* GenBank code HQ610440 and *Urospora wormskioldii* GenBank code HQ610441. Sequences used in the phylogenetic tree are listed in Table 1.

Comparison of recent species richness to historical findings

To assess the potential misidentification of historical specimens, historical vouchers of Ulvales taxa from the study area and neighbouring regions were obtained from several macroalgae collections and herbaria (Herbarium of the Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany (BRM), Herbarium of GEOMAR Helmholtz Centre for Ocean Research, Kiel, Germany (GEO); Herbarium of the Natural History Museum of Denmark, Copenhagen, Denmark (C)) and morphologically compared to specimens collected during the present study. The micro- and macromorphological characters of the vouchers were assessed using the above-mentioned criteria. When possible, small thallus pieces of the historical voucher specimens were sampled for molecular verification of species identity, as described in Steinhagen *et al.* (2018*a*).

Results

A total of 370 *Ulva sensu lato* samples were processed genetically for species discrimination and identification, on the basis of *tuf*A sequence data, and the full dataset was subject to phylogenetic analyses (see Supplementary table S1). In addition, an analysis with selected representative sequences was also performed (Fig. 2). The ML and BI analyses yielded congruent consensus trees. The species observed during the present study are described here, with a few particularly conspicuous species discussed in detail, and the majority are discussed in more depth in the Supplementary Information.

The phylogenetic analyses separated the investigated specimens into 20 taxonomic entities, nearly all of which could be resolved on the basis of peer-reviewed reference sequences provided by GenBank. More specifically, the taxa were identified as members of *Ulva, Umbraulva, Percursaria, Blidingia, Kornmannia, Monostroma* and *Protomonostroma*. One major branch within the consensus tree included members of *Ulva, Umbraulva* and *Percursaria* (i.e. the Ulvaceae) and was split into two subgroups, with the larger one containing *Ulva* taxa exclusively and the smaller subgroup containing several *Ulva* taxa (*U. lactuca, U. australis, U. intestinalis* and *U. compressa*), *Umbraulva dangeardii* and *Percursaria percursa*. However, this topology was only observed when *P. percursa* sequences were included; when *P. percursa* sequences were omitted, *Umbraulva* clustered as a sister group to *Ulva* (Supplementary fig. S1). Most of the species clades obtained full bootstrap and posterior probability support.

All *U. gigantea* sequences were identical to a sequence from New Brunswick, Canada (Fig. 2, Table 2). The specimens of this species were always represented by distromatic blades and were only found in a limited area in the Wadden Sea, except for one specimen in the Baltic Sea (for details see Supplementary Information).

Ulva rigida was always distromatic, and attached specimens were found in all three investigated regions, whereas mats of drifting specimens were only observed in the Wadden Sea (for details see Supplementary Information). The cluster representing *U. rigida* in our phylogenetic tree (Fig. 2) contained reference sequences for *Ulva laetevirens* Areschoug 1854 from Connecticut (JQ048942) and New Brunswick (HQ610428), as well as for *U. rigida* from the Italian Adriatic Sea (HE600178). All sequences were nearly identical, exhibiting divergences from the references of 0–0.26% and were placed in a well-delimited cluster.

Specimens that clustered most closely to *U. shanxiensis* type sequences were genetically more diverse than other taxa, with sequence distances ranging from 0 to 2.8% (Fig. 2). Furthermore, the specimens of *Ulva* sp. sampled during the present study were quite divergent (2.4–2.8%) from the *U. shanxiensis* type sequence. Specimens that belonged to this cluster were observed at all three main study areas and were typically found in areas of intense anthropogenic impact. The specimens were generally tubular but, nonetheless, of variable morphology (for details see Supplementary Information).

The clades delimiting the species *U. flexuosa* and *U. californica* received low to medium support. Both species were observed at all three main study areas. The more fragile and relatively rare species, *U. flexuosa*, exhibited only tubular morphologies and was generally observed unattached. The sequences of specimens in this clade differed from reference sequences from Canada (HQ610296) and South Korea (JN029309) by 0–0.17%. In contrast, the more robust of the two species, *U. californica*, exhibited more variable morphology, ranging from tubular to lanceolate or amorphous forms, and preferentially settled on artificial substrates. The sequences of specimens in this clade differed from reference sequences

Table 1. List of green algal samples collected in 2014–2016 in northern Germany and used in the displayed phylogenetic tree.

[a]Accession no.	Species	[b]Voucher no.	Collection date	Region	Location	[c]Site No.	Lat.	Long.
MH475471	*Ulva australis*	TD_10	24-Jul-2014	Wadden Sea	Norderfriedrichskoog	28	N 54,4136	E 8,8789
MH475472	*Ulva australis*	TD_34	15-Aug-2014	Wadden Sea	St. Peter-Ording	30	N 54,2857	E 8,7032
MH475473	*Ulva australis*	TD_36	16-Aug-2014	Wadden Sea	St. Peter-Ording	29	N 54,3267	E 8,5851
MH475450	*Ulva californica*	S_106	30-Jul-2014	Wadden Sea	Dagebuell	11	N 54,7301	E 8,6892
MH475454	*Ulva californica*	S_791	23-Sep-2015	Helgoland	Helgoland	48	N 54,1837	E 7,8886
MF979651	*Ulva compressa*	S_672	21-Apr-2015	Wadden Sea	Finkhaushallig	27	N 54,4156	E 8,9036
MF979652	*Ulva compressa*	S_514_B	19-Sep-2014	Baltic Sea	Wulfen	121	N 54,4089	E 11,1731
MF979645	*Ulva compressa*	S_14_B	22-Jul-2014	Helgoland	Helgoland	46	N 54,1882	E 7,8742
MH475451	*Ulva flexuosa*	S_257	18-Aug-2014	Baltic Sea	Kiel	78	N 54,3538	E 10,1413
MH475452	*Ulva flexuosa*	S_769	16-Aug-2015	Wadden Sea	Dagebuell	11	N 54,7301	E 8,6892
MH475453	*Ulva flexuosa*	S_794	23-Sep-2015	Helgoland	Helgoland	51	N 54,1780	E 7,8887
MH475474	*Ulva gigantea*	S_775	16-Aug-2015	Wadden Sea	Dagebuell	10	N 54,7304	E 8,6939
MH475475	*Ulva gigantea*	S_564	9-Apr-2015	Wadden Sea	Friedrich-Wilhelm-Luebke-Koog, Rhymschlot	7	N 54,8333	E 8,6142
MH475476	*Ulva gigantea*	S_632	17-Apr-2015	Wadden Sea	Dagebuell	11	N 54,7301	E 8,6892
MH475477	*Ulva intestinalis*	S_72	24-Jul-2014	Baltic Sea	Gluecksburg	55	N 54,8392	E 9,5176
MH475478	*Ulva intestinalis*	S_133	31-Jul-2014	Wadden Sea	Schluettsiel	13	N 54,6844	E 8,7539
MH475479	*Ulva lactuca*	S_729	24-Apr-2015	Helgoland	Helgoland	47	N 54,1882	E 7,8801
MH475480	*Ulva lactuca*	S_696	23-Apr-2015	Helgoland	Helgoland	50	N 54,1797	E 7,8896
MH475447	*Ulva linza 1*	S_241_U. linza 1	18-Aug-2014	Baltic Sea	Falckenstein	76	N 54,3904	E 10,1922
MH475448	*Ulva linza 1*	S_504_U. linza_1	16-Sep-2014	Wadden Sea	Hamburger Hallig	18	N 54,5990	E 8,8122
MH475449	*Ulva linza 1*	S_64_U. linza_1	24-Jul-2014	Baltic Sea	Gluecksburg	55	N54,8392	E 9,5176
MH475445	*Ulva linza 2*	S_727_U. linza_2	24-Apr-2015	Helgoland	Helgoland	52	N 54,1772	E 7,8930
MH475446	*Ulva linza 2*	S_8_U. linza_2	22-Jul-2014	Helgoland	Helgoland	46	N 54,1882	E 7,8742
MH475481	*Ulva prolifera*	S_196	12-Aug-2014	Baltic Sea	Falshoeft	60	N 54,7685	E 9,9653
MH475482	*Ulva prolifera*	S_9	22-Jul-2014	Helgoland	Helgoland	46	N 54,1882	E 7,8742
MH475483	*Ulva prolifera*	S_466	10-Sep-2014	Wadden Sea	Emmelsbuell	8	N 54,7949	E 8,6581
MH475484	*Ulva rigida*	S_449	9-Sep-2014	Wadden Sea	Friedrich-Wilhelm-Luebke-Koog, Rhymschlot	7	N 54,8333	E 8,6142
MH475485	*Ulva rigida*	S_123	30-Jul-2014	Wadden Sea	Dagebuell	11	N 54,7301	E 8,6892
MH475486	*Ulva rigida*	S_111	30-Jul-2014	Wadden Sea	Dagebuell	11	N 54,7301	E 8,6892
MH475487	*Ulva shanxiensis*	S_228	13-Aug-2014	Baltic Sea	Strande	71	N 54,4350	E 10,1702
MH475488	*Ulva shanxiensis*	S_269	18-Aug-2014	Baltic Sea	Moenkeberg	82	N 54,3465	E 10,1742
MH475489	*Ulva shanxiensis*	S_256	18-Aug-2014	Baltic Sea	Kiel	78	N 54,3538	E 10,1413
MH475490	*Ulva shanxiensis*	S_2_A	22-Jul-2014	Helgoland	Helgoland	53	N 54,1698	E 7,8894
MH475491	*Ulva shanxiensis*	S_317	22-Aug-2014	Baltic Sea	Sehlendorfer lake	95	N 54,3088	E 10,6886
MH475492	*Ulva shanxiensis*	S_221	13-Aug-2014	Baltic Sea	Strande	70	N 54,4362	E 10,1750
MH475493	*Ulva shanxiensis*	S_92	24-Jul-2014	Baltic Sea	Aschau	65	N 54,4608	E 9,9267
MH475494	*Ulva torta*	S_81	24-Jul-2014	Baltic Sea	Wackerballig	59	N 54,7586	E 9,8778
MH475495	*Ulva torta*	S_231	13-Aug-2014	Baltic Sea	Schilksee	72	N 54,4313	E 10,1693
MH475496	*Ulva torta*	S_350	25-Aug-2014	Baltic Sea	Heiligenhafen	99	N 54,3787	E 10,9555
MH475497	*Ulva torta*	S_73	24-Jul-2014	Baltic Sea	Gluecksburg	56	N 54,8368	E 9,5231
MH475498	*Umbraulva dangeardii*	R_1	8-Aug-2014	Helgoland	Helgoland	45	N 54,1874	E 7,8703
MH475499	*Umbraulva dangeardii*	R_2	8-Aug-2014	Helgoland	Helgoland	45	N 54,1874	E 7,8703
MH475464	*Blidingia marginata*	S_147_A	31-Jul-2014	Wadden Sea	Pellworm	21	N 54,4988	E 8,8087
MH475465	*Blidingia marginata*	S_577	14-Apr-2015	Wadden Sea	Brunsbuettel estuary	41	N 53,8890	E 9,1011
KT290281	*Blidingia minima*	DA_12	18-Jul-2013	Baltic Sea	Wohlenberg	*	N 53,9446	E 11,2444
MH475455	*Blidingia* sp. 1	S_828	24-Jul-2014	Wadden Sea	Schobuell	25	N 54,5079	E 8,9956
MH475456	*Blidingia* sp. 1	S_818	24-Jul-2017	Wadden Sea	Husum	26	N 54,4712	E 9,0280
MH475457	*Blidingia* sp. 1	S_815	24-Jul-2017	Wadden Sea	Finkhaushallig	27	N 54,4156	E 8,9036
MH475458	*Blidingia* sp. 1	S_813	24-Jul-2017	Wadden Sea	Friedrich-Wilhelm-Luebke-Koog	6	N 54,8374	E 8,6122
MH475459	*Blidingia* sp. 1	S_179	6-Aug-2014	Wadden Sea	Brunsbuettel estuary	41	N 53,8890	E 9,1011
MH475460	*Blidingia* sp. 2	S_34	23-Jul-2014	Helgoland	Helgoland	53	N 54,1720	E 7,8993
MH475461	*Blidingia* sp. 2	S_1	22-Jul-2014	Helgoland	Helgoland	48	N 54,1837	E 7,8886
MH475462	*Blidingia* sp. 2	S_39	23-Jul-2014	Helgoland	Helgoland	49	N 54,1825	E 7,8906
MH475463	*Blidingia* sp. 2	S_124	30-Jul-2014	Wadden Sea	Dagebuell	11	N 54,7301	E 8,6891
MH475466	*Kornmannia leptoderma*	S_154	5-Aug-2014	Wadden Sea	Finkhaushallig	27	N 54,4156	E 8,9036
MH475467	*Kornmannia leptoderma*	S_698	23-Apr-2015	Helgoland	Helgoland	50	N 54,1797	E 7,8896
MH475468	*Kornmannia leptoderma*	S_337	22-Aug-2014	Baltic Sea	Heiligenhafen	101	N 54,3795	E 10,9823

(*Continued*)

Table 1. (Continued).

[a]Accession no.	Species	[b]Voucher no.	Collection date	Region	Location	[c]Site No.	Lat.	Long.
MH475469	*Monostroma grevillei*	S_548	8-Apr-2015	Baltic Sea	Wulfen	121	N 54,4089	E 11,1731
MH475470	*Monostroma grevillei*	S_617	16-Apr-2015	Baltic Sea	Heiligenhafen	101	N 54,3795	E 10,9824
MH475500	*Percursaria percursa*	S_360	25-Aug-2014	Baltic Sea	Heiligenhafen	101	N 54,3795	E 10,9824
MH475501	*Protomonostroma undulatum*	S_733	24-Apr-2015	Helgoland	Helgoland	49	N 54,1825	E 7,8907

A sequence of *Blidingia minima* (KT290281) from a former survey at an adjacent site in Mecklenburg-Vorpommern is included as reference. [a] Voucher no. = Accession no. = GenBank accession number for *tuf*A gene. [b] Identification number assigned to the voucher specimen by the GEOMAR Helmholtz Centre for Ocean Research herbarium, Kiel, Germany. [c] Site no. = Referring to number in Fig 1.

from California (KM255003) and Canada (HQ610279 and HQ610280) by 0–1.82% (Fig. 2, Table 2).

Specimens that exhibited the relatively characteristic morphology of *Ulva torta* (long, narrow, with entangled unbranched tubular thalli and a central lumen of only 3–11 µm) were infrequently observed in either the Baltic or Wadden Seas and were not observed on Helgoland (for details see Supplementary Information). The specimens were clustered with reference sequences from southern Australia (JN029340) and British Columbia, Canada (HQ610437), but with only moderate support (85/0.96; Fig. 2), even though the specimens exhibited relatively low genetic divergence from the reference sequences (0.12–0.3%).

Sequences of the *U. linza* specimens exhibited strong genetic divergence (Fig. 2) and clustered in two strongly supported subgroups, *U. linza* 1 (100/1) and *U. linza* 2 (95/1). The *U. linza* 1 specimens were abundant over the whole study area (except Helgoland), formed a cluster with a genotype from Tasmania (JN029337) and exhibited very low sequence divergence (0–0.0014%). In contrast, the *U. linza* 2 specimens were exclusively collected from Helgoland, formed a cluster with a reference sequence from the North-east Pacific (KM254997) and exhibited slightly greater sequence divergence (0–0.49%). A historical herbarium voucher, originally identified as *Enteromorpha ahlneriana* Bliding nom. illeg., could be genetically assigned to *Ulva linza* sp. 1 (Fig. 2, Table 2). However, the phylogenetic differentiation was not reflected morphologically, and both the *U. linza* 1 and *U. linza* 2 specimens exhibited a wide spectrum of tubular to lanceolate and partly distromatic morphologies (for details see Supplementary Information).

The *U. prolifera* reference sequences from Manitoba, Canada (HQ610395) and Labrador, Canada (HQ610394) clustered with specimens from all three main study areas. However, our samples were clearly more similar to one another (–/0.98; Fig. 2), even though their genetic divergence from the reference ranged from 0 to 1.23%. *Ulva prolifera* was always attached when observed, was relatively abundant, exhibited a variety of tubular morphologies and frequently, but not always, possessed a characteristically twisted stipe-like base (for details see Supplementary Information).

Specimens that formed strongly supported clusters (99/1) with *U. lactuca* reference specimens from New Brunswick (HQ610341, 0–0.31% divergence) and California (KM255044, 0.12–0.47% divergence) were collected exclusively from Helgoland (Fig. 2). Even though Hughey et al. 2019 have suggested that the oldest available name for the European '*U. lactuca*' *is U. fenestrata*, we will refer to the here mentioned genotype as *U. lactuca* for reasons of general understanding. They exhibited distromatic thalli of various shapes and were characterized by relatively strong attachment. The specimens were found abundantly within the intertidal zone and grew attached to hard substrata, such as naturally occurring rocks (Fig. 3), stones and mussel beds or artificial breakwaters and piers. Only a few drifting specimens were observed and such drifting thalli exhibited clear indications of recent ruptures in the rhizoidal zone, thereby suggesting that drifting is not tolerated for long time periods. Specimens of this clade were never found in rockpools that were subject to potential desiccation or influence by rainwater. All thalli were distromatic throughout, and their shape varied from rounded to lobed (Fig. 4) or laciniate morphologies that could be straight, petiolate-like (Figs 5, 6) but also strongly curved (Fig. 7). Filled disc-like rhizoidal zones (Fig. 8, 13) were frequently observed at the base of the blade. The margins of the thalli were never toothed (grazing traces were clearly distinguished) and were usually smooth, although the rounded individuals sometimes exhibited ruffled margins. Holes (2–6 mm diameter) were observed infrequently. The thalli reached lengths of 40 cm and widths of 35 cm, but smaller individuals (max. 5 cm length and 2 cm width) were also observed. Longitudinal ridges were observed in the basal regions of most of the investigated specimens but were often absent in young thalli. The thalli were attached by obconically shaped stipe-like structures that terminated in broad rhizoidal zones (Figs 8, 9). The cells of the middle and apical thalli were arranged in curved or short rows, whereas cells of the laciniate thalli were

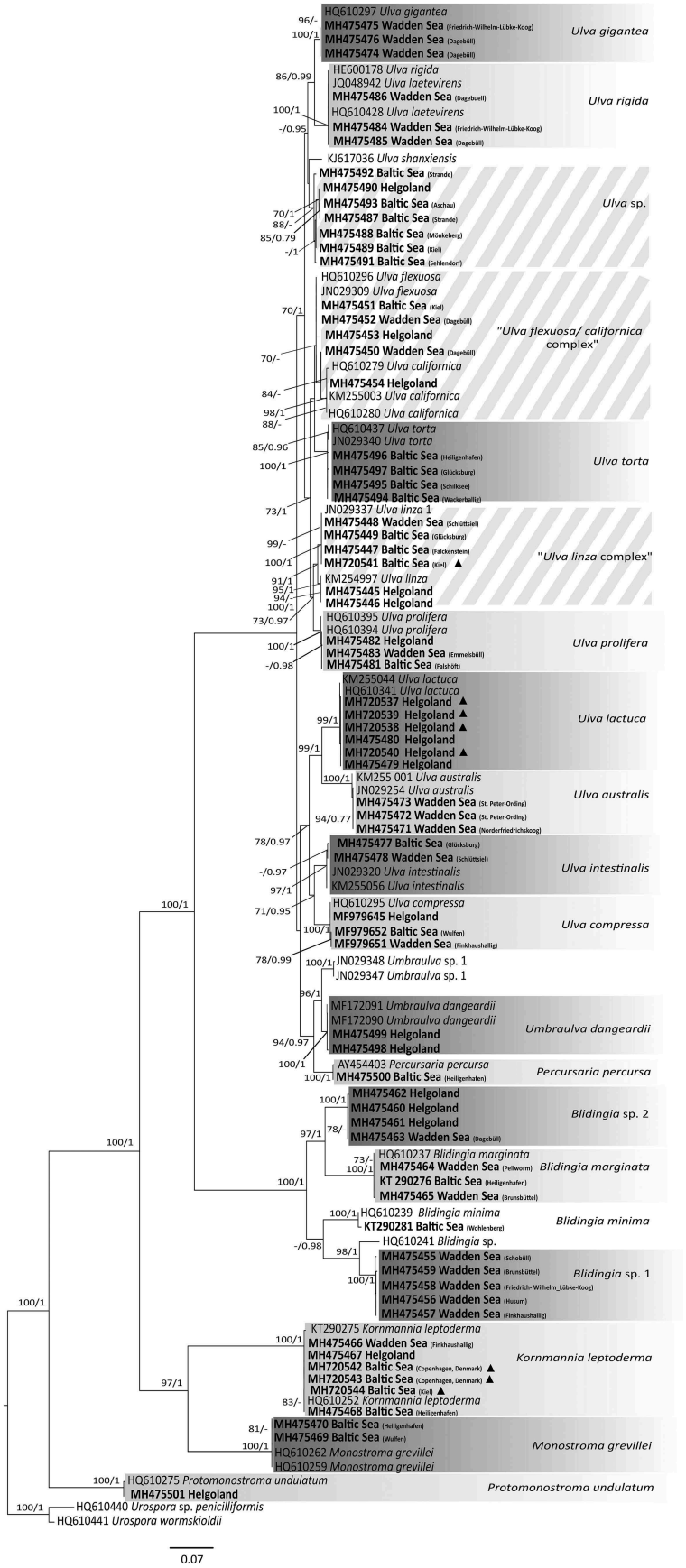


Fig. 2. Maximum likelihood phylogram of *tuf*A sequences from taxa of *Ulva sensu lato* from northern Germany. Solid triangles indicate herbarium vouchers (see also Table 2). The two shades of grey indicate clades that were present in the study area. Hatched boxes indicate species complexes and, thus, taxonomic entities that could not be clearly resolved phylogenetically. Numbers at nodes indicate bootstrap values (left) and Bayesian posterior probabilities (1000 replicates; right). Poorly supported nodes (< 70% bootstrap and < 0.70 Bayesian support) are not labelled. Branch lengths are proportional to sequence divergence.

Table 2. List of genetically processed herbarium material.

Region	Location	Collection date	[a]Herbar. ID	[b]Herbarium	Collector	[c]Accession no.	[d]Morphological identity	[e]Genetic identity *tufA*
Helgoland	Helgoland	10.04.1991	*BRM001700*	BRM	Kornmann and Sahling	MH720537	*Ulva pseudocurvata*	*Ulva lactuca*
Helgoland	Helgoland	10.04.1991	*BRM001703*	BRM	Kornmann and Sahling	MH720538	*Ulva pseudocurvata*	*Ulva lactuca*
Helgoland	Helgoland	22.10.1988	BRM007947	BRM	Kornmann and Sahling	MH720539	*Ulva pseudocurvata*	*Ulva lactuca*
Helgoland	Helgoland	17.07.1978	BRM007806	BRM	Kornmann and Sahling	MH720540	*Ulva tenera*	*Ulva lactuca*
Baltic Sea (Öresund)	Copenhagen	27.11.2007	73544	C	Ruth Nielsen and Peer Corfixen	MH720542	*Gayralia oxysperma*	*Kornmannia leptoderma*
Baltic Sea (Öresund)	Copenhagen	17.03.2004	40539	C	Ruth Nielsen and Peer Corfixen	MH720543	*Gayralia oxysperma*	*Kornmannia leptoderma*
Baltic Sea	Kiel, Friedrichsort	19.09.1962		GEO	Elfriede Kaminski	MH720544	*Gayralia oxysperma*	*Kornmannia leptoderma*
Baltic Sea	Kiel, Friedrichsort	30.9.1976	95	GEO	Elfriede Kaminski	MH720541	*Enteromorpha ahlneriana*	*Ulva linza*

[a] Herbar. ID = Barcode of respective Herbarium. [b] Herbarium = Abbreviation of the respective source herbaria (Herbarium of the Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany (BRM); Herbarium of GEOMAR Helmholtz Centre for Ocean Research, Kiel, Germany (GEO); Herbarium of the Natural History Museum of Denmark, Copenhagen, Denmark (C)). [c] Accession no. = GenBank accession number for *tufA* gene. [d] Morphological identity = Name that was historically assigned to specimen using morphological characters. [e] Genetic identity = Genetic identity obtained using analysis of *tufA*.

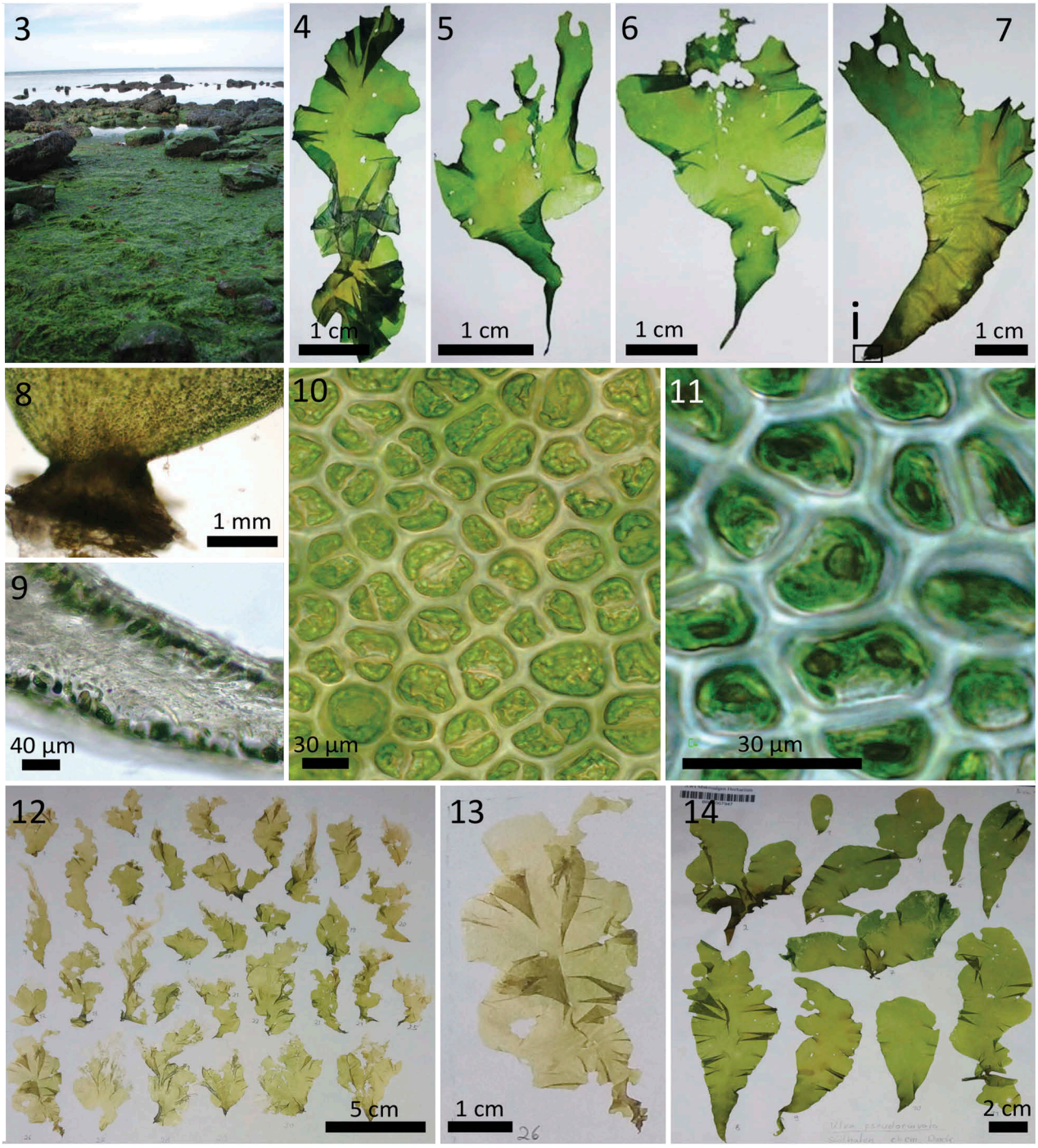
sometimes arranged in longitudinal rows. The cells were polygonal with rounded corners, 12–24 × 14–31 µm in surface view, and contained a parietal chloroplast (rarely filling the entire cell) and 1–2 (rarely up to 4) pyrenoids (Figs 10, 11). Cells of the rhizoidal zone contained up to seven pyrenoids, and both the stipe-like region and rhizoidal zone were filled by the elongated tails of the cell bodies, which became visible in microscopic transections. Thalli with these features were not only observed on Helgoland, but also along the mainland coasts, although they were always genetically assigned to *U. compressa*.

The sequence of one of over 100 syntypes of *U. tenera* Kornmann & Sahling – originating from Helgoland and stored at the BRM on Helgoland (voucher ID: BRM007806; Figs 12, 13, Table 2) – clearly clustered with *U. lactuca* (Fig. 2). Additionally, sequences from historical *U. pseudocurvata* vouchers (BRM001703 and BRM001700; Fig. 14), which provided the first evidence of the species' presence on Helgoland and were displayed in the publication of Kornmann & Sahling (1994), were also placed, with full bootstrap support, in the clade representing *U. lactuca* and were identical to the sequences of recently collected *U. lactuca* samples from Helgoland (Fig. 2).

A few specimens from the west coast of Helgoland and an area around the peninsula of Eiderstedt in the Wadden Sea (i.e. fully marine environments) were clustered with an *U. australis* reference sample from Australia (JN029254, genetic dissimilarity 0–0.33%, Fig. 2, Table 2). However, morphological comparison was impossible since only small thallus pieces conserved in silica gel could be obtained from our study area.

Sequences from the *U. intestinalis* specimens formed a fully supported clade (100/1) with reference specimens from Australia (JN029320) and California (KM255056; Fig. 2) but consistently exhibited slight divergence from both reference sequences (0.12–0.45%). The species was abundantly present at all three main study areas and in salinities that ranged from fresh water to fully marine. All of the specimens investigated exhibited tubular morphology, and most individuals were inflated and unbranched, corresponding to the typical morphology of *U. intestinalis*. Furthermore, specimens that exhibited branched and unbranched morphologies could not be distinguished genetically (for details see Supplementary Information).

Ulva compressa was abundant in all three main study areas, although only tubular, usually branched individuals were observed on Helgoland, whereas only distromatic sheet-like specimens were found along the Baltic coast. Both morphologies were encountered along the Wadden coast, sometimes even at the same location. Furthermore, individuals exhibiting transition forms between the two



Figs 3–14. Morphology of *Ulva lactuca* specimens from Helgoland, Germany. **Fig. 3.** *U. lactuca* population growing on the northeast rocky tidal flats. **Fig. 4.** Typical lobular morphotype. **Figs 5, 6.** Petiolate-like morphotype. **Fig. 7.** Strongly curved morphotype, with (**Fig. 8**) a disk-like rhizoidal zone (cross section) and (**Fig. 9**) elongated club-shaped cells that extend to the centre of the rhizoidal disc. **Fig. 10.** Cells of the apical and middle thallus parts, with a hood-shaped chloroplast and one (sometimes two) central or marginal pyrenoids. **Fig. 11.** Marginal pyrenoid. **Fig. 12.** *U. tenera* syntypes collected from Helgoland in 1978 (Herbarium of the Alfred Wegener Institute, Bremerhaven; ID BRM007806). (I) However, by sequencing one individual (see also Table 2 and Fig. 2), its genetic affiliation to *U. lactuca* was confirmed. **Fig. 14.** *U. pseudocurvata* specimens collected from Helgoland in 1988 (Herbarium of the Alfred Wegener Institute, Bremerhaven; ID BRM007947); arrowhead indicates specimen that was genetically identified as *U. lactuca* (see also Table 2 and Fig. 2).

morphologies were only rarely observed (for details see Supplementary Information). However, the morphotypes were not separated during the phylogenetic analyses and remained clustered with a *U. compressa* reference sample from Canada, New Brunswick (HQ610295, genetic dissimilarity 0–0.77%).

Sequences that were identical to those of *Umbraulva dangeardii* reference sequences from southern Italy (MF172090 and MF172091, genetic dissimilarity: 0–0.13%) were only recovered from specimens collected at Helgoland at a depth of 8 m. The specimens were distromatic sheets with a

conspicuous dark olive colour and thin, soft texture, which corresponds to descriptions of specimens from the British Isles (Maggs *et al.*, 2007*a*).

Percursaria percursa was only encountered once in a macroscopically visible state at Heiligenhafen (Baltic Sea), where it grew unattached in dense mats in the supralittoral zone. Microscopic examination confirmed the typical morphology of unbranched biseriate filaments (Maggs & Kelly, 2007) and our sequence was placed in a fully supported cluster with a *P. percursa* reference sequence (AY454403, genetic dissimilarity: 0.13%).

A well-delimited cluster that included *Blidingia marginata, B. minima* and *Blidingia* sp. reference sequences formed a sister clade to that including the *Ulva, Umbraulva* and *Percursaria* sequences and included four genetic entities (Fig. 2). Two of the four subgroups could not be resolved, since they did not match any references in GenBank, but were putatively identified as *Blidingia* specimens based on sequence similarity, overall morphology and growth habit.

Specimens that exhibited low genetic variability and clustered with a *B. marginata* reference sequence from New Brunswick, Canada (HQ610237, genetic dissimilarity: 0–0.28%) were abundant in all three main study areas. The specimens formed dense populations of variable tubular morphology in the upper intertidal and supralittoral zones and were often encountered as the only macroalgal settlers in microhabitats that are influenced by fresh water and that may fall dry for longer periods (for details see Supplementary Information).

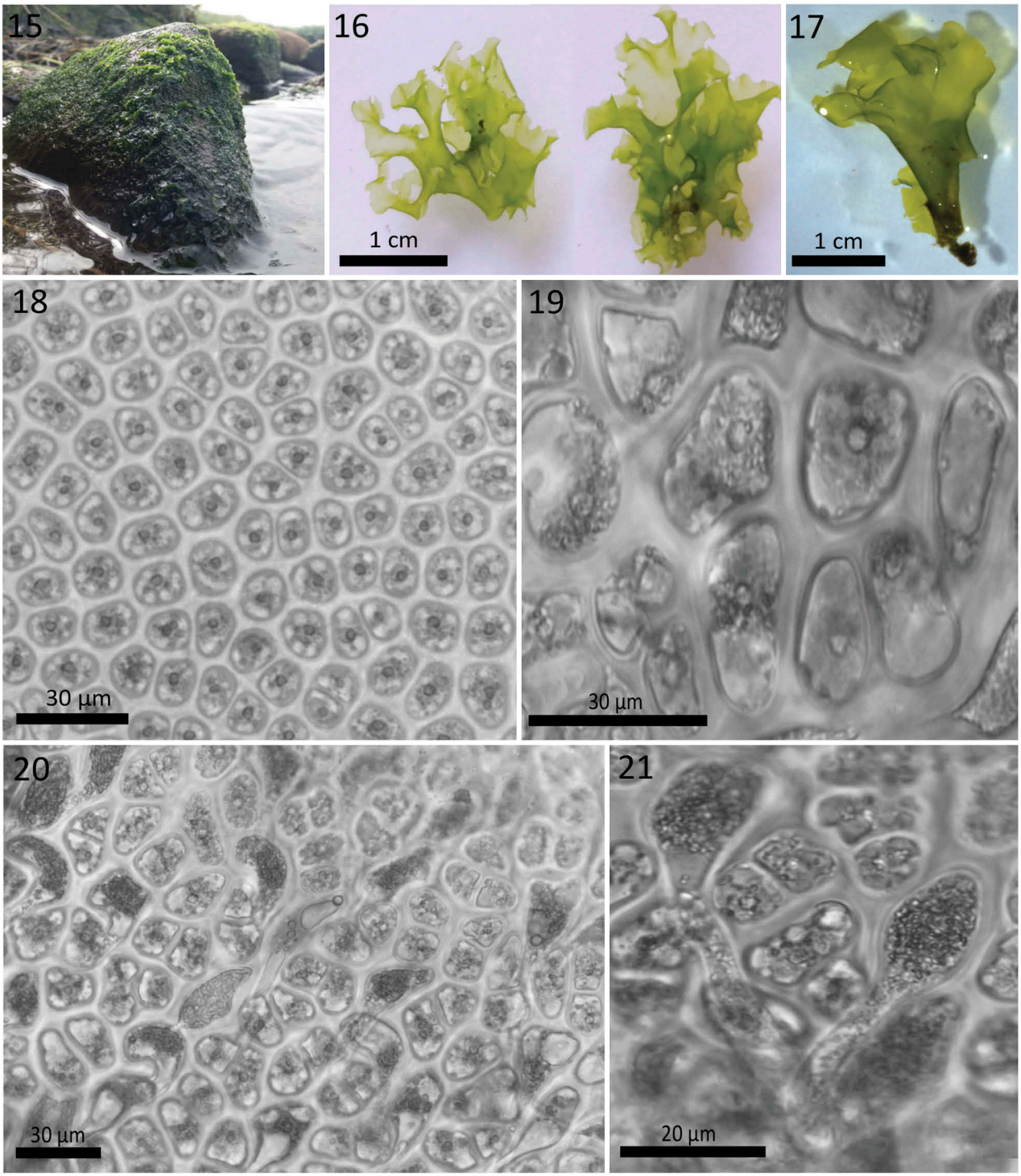
Specimens that belonged to the unresolved entity *Blidingia* sp. 2 were morphologically indistinguishable from *B. marginata* but formed a separate and fully supported cluster with 8–8.2% divergence from the *B. marginata* reference sequence (HQ610237). These specimens were collected from Helgoland and one site in the Wadden Sea (Dagebüll).

Molecular analysis failed to confirm the presence of *B. minima*. However, in a previous project *B. minima* was found at Wohlenberg (Fig. 1, site marked by an asterisk in general map), a site only 30 km to the East in the neighbouring German state of Mecklenburg-Vorpommern. A sample from the previously reported population was included in the phylogenetic tree (KT290281, Fig. 2) and formed a fully supported clade with a *B. minima* reference sequence from Canada (HQ610239).

Specimens that belonged to the unresolved entity *Blidingia* sp. 1 formed a fully supported cluster that was clearly delimited from other *Blidingia* species and genetically dissimilar (4.5–4.8%) from the *B. minima* reference sample (HQ610239). Specimens that belonged to this cluster had a broad distribution and were observed in all three main study areas, at remote and protected sites as well as in highly trafficked waters (see also Steinhagen *et al.*, 2018*b*). The specimens grew as mats on various substrata in the supralittoral zone and were often found in the close vicinity of freshwater inflows, such as drainage pipes. The *Blidingia* sp. 1 specimens were relatively minute and they often exhibited a characteristic antler-like branched tubular morphology. However, macroscopically visible branches were rarely observed and appeared as spiralled or inflated.

The remaining three clades (Fig. 2) only included specimens with monostromatic blades and formed fully supported clusters with reference sequences of *Kornmannia leptoderma, Monostroma grevillei* and *Protomonostroma undulatum. Kornmannia leptoderma* specimens from all three study areas clustered with reference sequences from Canada (HQ610252, dissimilarity: 0–0.38%) and northern Germany (KT290275) and exhibited little to no genetic diversity. Specimens that belonged to this cluster were found in the middle and lower intertidal zones and were typically attached to substrata. The specimens appeared to avoid exposure to direct sunlight and were frequently found on the shaded sides of stones (Fig. 15) or jetties, or under piers. The 1–5 cm (rarely up to 8 cm) long thalli of the *K. leptoderma* specimens were nearly unrecognizable when the substrate became dry during low tide. The membranous and very soft thalli appeared funnel-shaped (Fig. 17), lanceolate or rosette-like (Fig. 16). Older thalli that had sporulated were amorphous in shape and deeply cut. The rhizoidal zone was not defined by a disc-like structure, and cells proceeded without tapering in a stipe-like region. Cells in the apical and middle thallus parts differed in shape from those in the basal thallus parts. The cells of the upper and middle thallus regions were either polygonal to round or with sharp and clearly defined angular edges, 9–15 × 11–16 μm in surface view, with a single centrally located pyrenoid and a chloroplast that was either marginal or filling the entire cell (Figs 18, 19). Meanwhile, the cells of the apical and middle thallus regions were thick-walled (Fig. 19), although progressively thinner and larger toward the basal region, and with 1–3 (rarely 4) pyrenoids per cell. In the lower mid-thallus parts, the cells were 11–21 × 11–27 (32) μm in surface view and sometimes appeared grainy, whereas others already resembled rhizoidal cells with long drawn-out tips (Figs 20, 21). Cells of the rhizoidal zone were up to 50 μm long, always grainy, with rhizoidal tips extending from the main body, and typically with 1–3 pyrenoids (rarely more; Fig. 21).

All the *M. grevillei* specimens formed a cluster and were often identical to reference sequences from Maine, USA (HQ610262, dissimilarity: 0–0.51%) and New Brunswick, Canada (HQ610259, dissimilarity: 0–0.39%). The species was abundant in the Baltic Sea and



Figs 15–21. Morphology of *Kornmannia leptoderma* specimens from northern Germany. **Fig. 15.** Typical sampling site along the coast of the Baltic Sea (Aschau lagoon), with *K. leptoderma* growing on the shaded side of a rock. **Fig. 16.** Rosette-shaped specimens from the Baltic Sea (Aschau lagoon). **Fig. 17.** Funnel-shaped specimen from the Baltic Sea (coastal inlet, Schlei, Lindaunis). **Fig. 18.** Cells in apical and middle thallus regions, in rows or pairs. **Fig. 19.** Marginal chloroplasts and central pyrenoids (one or rarely two). **Figs 20, 21.** Club-shaped cells of the rhizoidal zone.

also occurred on Helgoland. However, the species was only observed during spring (March to May), and in late spring drifting mats of *M. grevillei* frequently developed in sheltered bays, harbours and lagoons. The cells of the *M. grevillei* specimens were arranged in more distinct rows than those of the *K. leptoderma* specimens.

The only specimen that clustered with the *P. undulatum* reference sequence (dissimilarity: 0.13%) was found in the lower intertidal zone of Helgoland. As in *M. grevillei*, the cells were often arranged in rows. However, instead of a smooth transition from basal cells to rhizoidal cells, abrupt changes in cell shape were observed, and the rhizoidal cells were longer than those of the *K. leptoderma* specimens (60–90 µm, up to 110 µm; Supplementary fig. S2; for details see Supplementary Information).

An additional taxon that might represent *Gayralia oxysperma* was not detected at any of the study sites, even though Kützing (1843) originally described its basionym, *Ulva oxysperma*, on the basis of material collected in Schleswig-Holstein at Winning (located at the inner Schlei, a narrow inlet of the Baltic Sea, site 127 in Fig. 1). Unfortunately, the type material of *U. oxysperma* appears to be lost. However, historical *G. oxysperma* vouchers from Friedrichsort, Kiel, Germany, that were sampled in 1962 (MH720544) and from Copenhagen, Denmark, that were sampled in 2004 and 2007 (MH720542 and MH720543) were available for sequencing. Notably, all three voucher sequences clustered with *K. leptoderma* (Fig. 2, Table 2). During subsequent visits (i.e. additional collections in 2017 and 2018), thalli exhibiting the described morphology of *G. oxysperma* were not detected at Winning (site 127 in Fig. 1, salinity 1) but were collected at the inner Schlei at Brodersby (site 128 in Fig. 1, salinity 7), which is 10 km from Winning, and at Lindaunis (site 61 in Fig. 1, Supplementary table S1), which is 30 km from Winning. However, sequences from these *G. oxysperma*-like specimens were also placed in the *K. leptoderma* cluster (MH720545–MH720547; Supplementary table S1).

Discussion

The 20 taxa of green algae that were detected in the present study (Fig. 2) can be identified with variable degrees of certainty. Only one taxon could be assigned to a clade that included a reference sequence from type material, namely for *Ulva tenera* (Kornmann & Sahling, 1994). However, *U. tenera* was described relatively recently, and the corresponding cluster in our phylogenetic analysis also encompassed several reference sequences from specimens that were recognized elsewhere as *U. lactuca* L. Even though only one of the more than 100 *U. tenera* syntypes was examined, and given that the current concept of *U. lactuca* has been challenged (Butler, 2007), the phylogenetic analysis presented here strongly suggests that *U. tenera* is a synonym of *U. lactuca*. This view is further supported by the observation that young *U. lactuca* specimens from Helgoland exhibit the described morphology of *U. tenera* (Kornmann & Sahling, 1994). Other described characteristics of *U. tenera* are its restriction to the uppermost eulittoral and its exclusively vegetative propagation (i.e. with biflagellate spores; Kornmann & Sahling, 1994); apparently, the authors observed dwarfish forms of *U. lactuca* that were adapted to extended air exposure. Therefore, *U. tenera* is here reduced to synonymy with *U. lactuca*. Other homotypic synonyms of *U. lactuca* cited below are according to Guiry & Guiry (2018).

Ulva lactuca

Linnaeus, C. 1753. Species plantarum, exhibentes plantas rite cognitas, ad gen era relatas, cum differentiis specificis, nominibus trivialibus, synonymis selectis, locis natalibus, secundum systema sexuale digestas. Vol. 2 pp. [i], 561–1200, [1–30, index], [i, err.]. Holmiae [Stockholm]: Impensis Laurentii Salvii.

Homotypic synonyms:
Phyllona lactuca (Linnaeus) F.H.Wiggers 1780
Monostroma lactuca (Linnaeus) J.Agardh 1883
Ulva tenera Kornmann & Sahling 1994

The genetic-based species identities of several other taxa corresponded to characteristic morphological traits. Indeed, this was the case for *U. torta*, which usually formed massively intertwined tubular thalli of small diameter; *U. prolifera*, which mostly exhibited characteristically twisted stipes; and *Umbraulva dangeardii*, which is characterized by its olive green pigmentation. Meanwhile, the genetic-based species identities of the three monostromatic taxa corresponded to known phenotypic traits. For example, *M. grevillei* was only observed during spring, which was not the case for any other entity; *K. leptoderma* exhibited a characteristic heteromorphic life cycle, as reported elsewhere (Weinberger *et al.*, 2018); and *P. undulatum*, despite only being observed once, exhibited a typical morphology (see Supplementary Information). Furthermore, specimens that clustered with *Ulva intestinalis* mostly exhibited the tubular and unbranched morphology considered characteristic of the species (Kornmann & Sahling, 1977; Rothmaler, 1984; Pankow, 1990), but branched specimens were occasionally observed, as reported previously (Reed & Russell, 1978; Blomster *et al.*, 1998), probably promoted by low salinity (Steinhagen *et al.*, 2018*b*).

However, for most cases, genetic-based species identities failed to correspond to characteristic morphological traits. For example, specimens that exhibited the characteristic lanceolate and partly distromatic type morphology of *U. linza* (Kornmann & Sahling, 1977; Rothmaler, 1984; Pankow, 1990) clustered with *U. linza* reference sequences, but also with sequences from specimens that exhibited tubular and branched morphologies corresponding to descriptions of *U. procera* and *U. ahlneriana* (Kornmann & Sahling, 1977; Rothmaler, 1984; Pankow, 1990) and a sequence from a historical voucher of *U. ahlneriana* (Fig. 2). This observation supports the previous suggestions that *U. procera* (Maggs *et al.*, 2007*b*) and *U. ahlneriana*(Guiry & Guiry, 2018) are synonyms of *U. linza*. It was interesting that the cluster representing *U. linza* in our phylogenetic tree included two lineages which were morphologically

indistinct. One of the lineages was only detected on Helgoland, whereas the second was only detected on mainland coasts. However, more information is needed to determine whether the groups represent distinct species or simply unique genotypes that developed in response to geographic separation.

Meanwhile, the *U. compressa* specimens also exhibited multiple gross morphologies. One morphotype was only observed on North Sea coasts and corresponded to the morphology of the tubular and branched type material (Linnaeus, 1753). However, as already discussed elsewhere (Steinhagen *et al.*, 2018*a*), genetically indistinguishable specimens from the Baltic and Wadden Seas exhibited a completely different morphology that was consistently distromatic and sheet-like. Evidently, the distromatic morphology of *U. compressa* strongly overlaps with the allegedly unique morphology of *U. lactuca*, thereby causing a considerable amount of historical taxonomic confusion (Steinhagen *et al.*, 2018*a*). Based on the results of the present study, *U. lactuca* in northern Germany is only present on Helgoland (Table 2, Supplementary table 2), whereas historical records from the Baltic Sea (Schories *et al.*, 2009) are misidentified *U. compressa* specimens (Steinhagen *et al.*, 2018*a*). Notably, historical vouchers from Helgoland (Kornmann & Sahling, 1994) that exhibited the curved morphology of *U. pseudocurvata* (Hoeksema & Van den Hoek, 1983) yielded sequences that clustered with *U. lactuca* sequences, whereas specimens that were recently collected from mainland coasts of northern Germany (Steinhagen *et al.*, 2018*a*) and elsewhere (Tan *et al.*, 1999; Hayden & Waaland, 2004) exhibiting the same morphology yielded sequences that clustered with *U. compressa* sequences. This clearly challenges the validity of *U. pseudocurvata* as a taxonomic entity, because its description is based on morphological traits that are clearly not specific, and it also confirms the strong morphological plasticity of *U. lactuca* on Helgoland and *U. compressa*, in its distromatic form, on the mainland coasts of northern Germany.

In addition to *U. lactuca* and the distromatic form of *U. compressa*, three additional entities with consistently distromatic blades were also observed in the present study. These specimens clustered with *U. gigantea, U. australis, U. rigida* and *U. laetevirens* reference specimens (Fig. 2). In these cases, the observed morphologies generally paralleled the corresponding type morphologies. However, based on morphological observations the taxa were not reliably distinguishable from one another, *U. lactuca*, or the distromatic form of *U. compressa*. Furthermore, as recently demonstrated by ITS and *rbc*L analysis (Horta *et al.*, 2018), *U. rigida* and *U. laetevirens* could not be distinguished using *tuf*A gene sequences. Therefore, *U. laetevirens* Areschoug 1854 should be considered a synonym of *U. rigida* C. Agardh 1823.

Some clades with tubular morphologies could not be clearly resolved. As reported previously (Heesch *et al.*, 2009; Kraft *et al.*, 2010; Saunders & Kucera, 2010; Kirkendale *et al.*, 2013), there was no clear species boundary between *U. flexuosa* and *U. californica* (Fig. 2). However, despite this observation, Hiraoka *et al.* (2017) used hybridization experiments to confirm the biological separation of *U. flexuosa* and *U. californica* from Japan. Because cross-breeding experiments were not included in the present study, we have chosen to indicate the species' lack of genetic resolution using the term '*Ulva flexuosa/californica* complex'.

A reference sequence for type material of the tubular species *U. shanxiensis*, which was recently described from a freshwater stream in northern China (Chen *et al.*, 2015), was placed basal to a clade of tubular specimens in the phylogenetic analysis of the present study (Fig. 2). However, the clade encompassing *U. shanxiensis* and the tubular specimens was poorly supported, indicating relatively high sequence divergence (Fig. 2, note branch length). Therefore, the tubular specimens are unlikely to belong to *U. shanxiensis*, and the identity of the clade remains unidentified as a result.

Identities could also not be determined for two genetic entities in the genus *Blidingia* (*Blidingia* sp. 1 and *Blidingia* sp. 2), since they did not match any available reference sequences. Specimens of the *Blidingia* sp. 2 clade exhibited strong morphological overlap with a second clade encompassing a reference sequence of *Blidingia marginata* and could only be distinguished molecularly. The morphology of both clades was consistent with that of *B. marginata* but, perhaps, also with that of *B. ramifera* (Garbary & Barkhouse, 1987), a species that has not yet been reported from the area and which is, for formal reasons, invalid (Cormaci *et al.*, 2014) and currently regarded as a synonym of *B. marginata* (Guiry & Guiry, 2018). In contrast, specimens of the relatively abundant *Blidingia* sp. 1 exhibited unique genetic and morphological traits that clearly distinguished them from other *Blidingia* taxa in northern Germany. In addition to *B. marginata* and *B. minima*, two other *Blidingia* species (*B. chadefaudii* and *B. subsalsa*) have also been reported from the German coasts of the North Sea (Kornmann & Sahling, 1978; Bartsch & Kuhlenkamp, 2000; Schories *et al.*, 2009). However, no molecular reference data were available for *B. chadefaudii* and *B. subsalsa*, and morphological identification criteria for the species remain ambiguous and overlapping. Therefore, in order to identify *Blidingia* sp. 1 and *Blidingia* sp. 2 and to confirm the identities of *B. marginata* and *B. minima*, type material of different *Blidingia* species

should be analysed by molecular markers and species life cycles should be documented using cultivated material. The same strategy might also facilitate the identification of ambiguous *Ulva* specimens in the future.

Notably, our phylogenetic analyses did not support the monophyly of the genus *Ulva* (Fig. 2). In our study the inclusion of *U. lactuca*, *U. australis*, *U. intestinalis* and *U. compressa* as a sister clade of *Umbraulva* species and *Percursaria percursa* was revealed (Fig. 2), in contrast to previous studies which used other marker genes (Hayden *et al.*, 2003; Heesch *et al.*, 2009; Kirkendale *et al.*, 2013). This topology was not observed when *P. percursa* was excluded from the analysis (Supplementary fig. S1). However, the inclusion of more, rather than fewer, taxa is more likely to yield true phylogenetic relationships.

The species inventory of *Ulva sensu lato* of the present study diverged considerably from the expected inventory (Schories *et al.*, 2009). Four species (*U. australis, U. californica, U. gigantea* and *Umbraulva dangeardii*) were observed in the area for the first time (Fig. 22). *Ulva australis* was first introduced to southern France and very recently reported from the Dutch Oosterschelde estuary (Fort *et al.*, 2019). Now, the species is also present in the North Friesian Wadden Sea. The same is true for *U. gigantea*, which, in Europe, had only been reported from Britain and other westerly locations (Maggs *et al.*, 2007*b*). Single individuals of *U. californica* were first observed in Germany in 2008 on the Wadden Sea island of Wangerooge in Lower Saxony (Lackschewitz *et al.*, 2015) and, over the next six years, eventually reached the SW Baltic Sea. In the present study, *Umbraulva dangeardii* was only observed at one site on Helgoland (Table 2, Supplementary table S1). It is interesting that even though Helgoland is a phycological hotspot in Germany, *U. dangeardii* has never before been included in inventories (Kornmann & Sahling, 1977, 1983, 1994; Bartsch & Kuhlenkamp, 2000), suggesting recent introduction. Yet, the presence of *U. dangeardii* in Germany may have been ignored for some time, due to the preference of the species for subtidal habitats. In addition to the above-mentioned newly introduced species, three (*Blidingia* sp. 1, *Blidingia* sp. 2, *Ulva* sp.) or even four (if one of the two genetic entities within *U. linza* is included) additional taxa that were observed in our study probably represent cryptic and perhaps undescribed species that have so far not been recognized.

Despite these new records, the morphology-based species inventories of all three main study areas were expected to be larger than the genetically validated ones (Fig. 22). Altogether, 14 of the species (members of *Ulva, Blidingia, Monostroma, Gayralia* and *Ulvaria*) that were listed by Schories *et al.* (2009) and are currently accepted taxonomically (Guiry & Guiry, 2018) were not encountered genetically in the present study. This lack of detection could indicate their absence but might also be attributed to other factors, such as low abundance or lack of molecular reference material. Indeed, no *tufA* reference sequences are available for 11 of the 14 missing species, and the numerous historical records from the area may be the result of misidentification and taxonomic confusion.

As discussed above, records of *Ulva pseudocurvata* from northern Germany are often, and perhaps always, due to the misidentification of either *U. compressa* or *U. lactuca*. Also, the only record of *U. splitiana* from our area (as *Enteromorpha jugoslavica*; Kaminski, 1980) was due to the misidentification of *U. linza*, as demonstrated through sequencing of the ITS marker gene from the corresponding herbarium voucher (Gesche Bock, pers. comm.). Furthermore, analysis of historical *Gayralia oxysperma* vouchers from northern Germany and adjacent areas indicated that all the vouchers were genetically identical to *Kornmannia leptoderma*, which had until now been considered a relatively rare species that was only present on Helgoland (Kornmann & Sahling, 1983) and, therefore, has not been included in identification keys for other parts of Germany (Rothmaler, 1984; Pankow, 1990) or adjacent areas (Brodie *et al.*, 2007). However, *K. leptoderma* was present in all three main areas of the present study (see also Weinberger *et al.*, 2018). In striking contrast, *G. oxysperma* was not observed, even at the type locality of its basionym *U. oxysperma* Kützing (see the Supplementary Information for a description of the relatively complicated nomenclatural history of *G. oxysperma*). For further details see Doty (1947), Gayral (1965) and Womersley (1984). This apparent absence or rarity of *G. oxysperma* is surprising because the species should be present across the entire Baltic Sea (Schories *et al.*, 2009). Descriptions of *G. oxysperma* (Rothmaler, 1984; Pankow, 1990) are in complete agreement with the morphology of *K. leptoderma* in our area (Figs 15–21, see also Weinberger *et al.*, 2018). The two species have very different life cycles (Vinogradova, 1969), but ontogenetic observations are time consuming, and for this reason most historical records of *G. oxysperma* are probably based on the morphological traits of field-collected material. As a consequence, it is likely that most records of *G. oxysperma* are due to the misidentification of *K. leptoderma*. Similarly, the molecular analysis of *G. oxysperma*-like specimens from the North-west Atlantic yielded two clusters attributed to

Species	Baltic Sea		Wadden Sea		Helgoland	
	2019	2009	2019	2009	2019	2009
Blidingia marginata (J.Agardh) P.J.L.Dangeard ex Bliding 1963	✓	✓	✓	✓	✓	✓
Blidingia minima (Nägeli ex Kützing) Kylin 1947	X	✓	X	✓	X	✓
Blidingia chadefaudii (Feldmann) Bliding 1963					X	✓
*Blidingia subsalsa (*Kjellmann) Kornmann & Sahling ex Scagel et al. 1989					X	✓
Blidingia sp. 1	✓	X	✓	X	✓	X
Blidingia sp. 2					✓	X
*Kornmannia leptoderma (*Kjellmann) Bliding 1969	✓	X	✓	✓	✓	✓
Ulva compressa Linnaeus 1753	✓	✓	✓	✓	✓	✓
Ulva pseudocurvata Koeman & Hoek 1981					X	✓
Ulva curvata (Kützing) De Toni 1889			X	✓		
Ulva flexuosa Wulfen 1803	✓	✓	✓	✓	✓	✓
Enteromorpha flexuosa subsp. *linziformis* (Bliding) Bliding 1963			X	✓		
Ulva flexuosa subsp. *paradoxa* (C. Agardh) M.J. Wynne 2005			X	✓	X	✓
Ulva californica Wille 1899	✓	X	✓	X	✓	X
Ulva intestinalis Linnaeus 1753	✓	✓	✓	✓	✓	✓
Ulva lactuca Linnaeus 1753	X	✓	X	✓	✓	✓
Ulva tenera Kornmann & Sahling 1994			X	✓	X	✓
Ulva linza Linnaeus 1753	✓	✓	✓	✓	✓	✓
Enteromorpha jugoslavica Bliding	X	✓				
Ulva prolifera O. F. Müller 1778	✓	✓	✓	✓	✓	✓
Ulva torta (Mertens) Trevisan 1841	✓	✓	✓	✓		
Ulva lobata (Kützing) Harvey 1855					X	✓
Ulva radiata (J.Agardh) H.S.Hayden, Blomster, Maggs, P.C.Silva, M.J.Stanhope & J.R.Waaland 2003			X	✓		
Ulva ralfsii (Harvey) Le Jolis 1863			X	✓		
Ulva simplex (K.L.Vinogradova) H.S.Hayden, Blomster, Maggs, P.C.Silva, M.J.Stanhope & J.R.Waaland 2003	X	✓	X	✓		
Ulva clathrata (Roth) C. Agardh 1811	X	✓	X	✓		
Ulva rigida C. Agardh 1823 and *Ulva scandinavica* Bliding 1968	✓	X	✓	✓	✓	X
Ulva gigantea (Kützing) Bliding 1969	✓	X	✓	X		
Ulva australis Areschoug 1854			✓	X	✓	X
Ulva sp.	✓	X	✓	X	✓	X
Umbraulva dangeardii M.J.Wynne & G.Furnari 2014					✓	X
Ulvaria fusca (Postels & Ruprecht) Vinogradova 1967	X	✓				
Percursaria percursa (C. Argardh)Rosenvinge 1893	✓	✓	X	✓	X	✓
Monostroma grevillei (Thoret) Wittrock 1866	✓	✓	X	✓	✓	✓
Monostroma arcticum Wittrock1866					X	✓
Protomonostroma undulatum (Wittrock) Vinogradova 1969					✓	✓
Gayralia oxysperma (Kützing) K.L. Vinogradova ex Scagel et al. 1989	X	✓				

Fig. 22. Comparison of molecular (*tuf*A)-based identification from the present study and the inventory list from Schories *et al.* (2009). List of species predicted by Schories *et al.* (2009) and detected in the present study (2019) from the Baltic Sea, Wadden Sea and Helgoland. **X**, species not observed; □, species observed; empty, unexpected by Schories *et al.* (2009) and not observed in present study. Light grey shading indicates agreement between Schories *et al.* (2009) and the present study, whereas dark grey shading indicates disagreement. For additional annotations or taxonomic notes by other authors see also Supplementary table S2.

Monostroma grevillei (Saunders & Kucera, 2010). Therefore, a thorough taxonomic reassessment of *G. oxysperma* and its populations is urgently needed.

Some species that were reported to occur only in parts of northern Germany were found to have broader distributions than expected. For instance, *U. rigida*, which was only expected to occur in the Wadden Sea and on Helgoland, was also observed in the Baltic Sea (Table 2, Supplementary table S1), and *K. leptoderma*, which had only been reported to occur on Helgoland, was observed in both the Baltic and Wadden Seas (Table 2, Supplementary table S1).

In summary, the current morphological concepts that are used for the identification of *Ulva* species and related taxa in northern Germany are neither in agreement with the species inventory of the area nor with the actual morphology of species that are present. Past morphological descriptions of *U. linza*, *U. intestinalis* and *U. compressa* have been too restrictive, thereby resulting in frequent misidentifications of these abundant taxa. Furthermore, several cryptic and/or newly introduced species, including *K. leptoderma*, *U. australis*, *U. californica*, *U. gigantea* and *Protomonostroma undulatum*, are now present in northern Germany, and several genetic entities, namely *Ulva* sp., *Blidingia* sp. 1 and *Blidingia* sp. 2, have yet to be identified. Meanwhile, *B. minima* and *G. oxysperma* were either absent or much rarer than expected, and certain other taxa that were expected in the area, namely *U. tenera*, are actually synonyms. The observations of the present study provide a basis for the development of improved identification keys, although it is unlikely that it will be possible to distinguish all species morphologically owing to the considerable overlap of traits. The DNA barcoding approach used in the present study clearly provides better resolution. However, *U. californica* and *U. flexuosa* cannot be clearly distinguished using the analysis of *tuf*A alone and more sequences of type material will be needed to improve the identification of species in the future. Furthermore, additional genetic markers should be investigated and cultivation studies should be performed to resolve remaining issues, such as the taxonomic affiliation of the newly found *Blidingia* species or relations among the genera *Ulva*, *Umbraulva* and *Percursaria*.

Acknowledgements

We would like to express gratitude to T. Dolch, K. Reise and R. Kuhlenkamp for support in specimen collection; the herbaria and macroalgae collections for granting us access to their herbaria and vouchers; our colleague D. Afanasyev for translating the Russian publications cited in our article; and G. Bonthond for valuable comments on the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

Supplementary information

Supplementary Information. (1) Distribution and specific characteristics of observed species, (2) nomenclatural history of *Gayralia oxysperma* and (3) seasonal species variation.
Supplementary table S1. Full collection data.
Supplementary table S2. Comparison of molecular (*tuf*A)-based identification from the present study and the inventory list from Schories *et al.* (2009).
Supplementary fig. S1. Maximum likelihood phylogram of *tuf*A sequences from taxa of *Ulva sensu lato* from northern Germany.
Supplementary fig. S2. Morphology of *Protomonostroma undulatum* specimens from Helgoland, Germany.

Author contributions

S. Steinhagen: experimental design, fieldwork and algae collection, laboratory work, macro- and microscopic observation, phylogenetic analysis, drafting and editing manuscript; R. Karez: experimental design, algae collection, drafting and editing manuscript; F. Weinberger: original concept, collection of specimens, drafting and editing manuscript.

References

Bartsch, I. & Kuhlenkamp, R. (2000). The marine macroalgae of Helgoland (North Sea): an annotated list of records between 1845 and 1999. *Helgoland Marine Research*, **54**: 160–189.

Blomster, J., Maggs, C.A. & Stanhope, M.J. (1998). Molecular and morphological analysis of *Enteromorpha intestinalis* and *E. compressa* (Chlorophyta) in the British Isles. *Journal of Phycology*, **34**: 319–340.

Blomster, J., Back, S., Fewer, D.P., Kiirikki, M., Lehvo, A., Maggs, C.A. & Stanhope, M.J. (2002). Novel morphology in *Enteromorpha* (Ulvophyceae) forming green tides. *American Journal of Botany*, **89**: 1756–1763.

Brodie, J., Maggs, C.A., Society, B.P. & John, D.M. (2007). *The Green Seaweeds of Britain and Ireland*. British Phycological Society, London.

Butler, D. (2007). Ancient algal mixup sorted. *Nature*. https://www.nature.com/news/2007/071220/full/news.2007.396.html.

Charlier, R.H., Morand, P., Finkl, C.W. & Thys, A. (2007). Green tides on the Brittany coasts. *Environmental Research, Engineering and Management*, **3**: 52–59.

Charlier, R.H., Morand, P. & Finkl, C.W. (2008). How Brittany and Florida coasts cope with green tides. *International Journal of Environmental Studies*, **65**: 191–208.

Chen, L., Feng, J. & Xie, S.-L. (2015). *Ulva shanxiensis* (Ulvaceae), a new species from Shanxi, China. *Novon*, **23**: 397–405.

Cormaci, M., Furnari, G., & Alongi, G. (2014). Flora marina bentonica del Mediterraneo: Chlorophyta. *Bollettino*

dell'Accademia Gioenia di Scienze Naturali di Catania, **47**: 11–436

Doty, M.S. (1947). The marine algae of Oregon. Part 1. Chlorophyta and Phaeophyta. *Farlowia*, **3**: 1–65.

Famà, P., Wysor, B., Kooistra, W.H.C.F. & Zuccarello, G.C. (2002). Molecular phylogeny of the genus *Caulerpa* (Caulerpales, Chlorophyta) inferred from chloroplast *tuf*A gene. *Journal of Phycology*, **38**: 1040–1050.

Fort, A., Lebrault, M., Allaire, M., Esteves-Ferreira, A.A., McHale, M., Lopez, F., Fariñas-Franco, J.M., Alseekh, S., Fernie, A.R. & Sulpice, R. (2019). Extensive variations in diurnal growth patterns and metabolism among *Ulva* spp. strains. *Plant Physiology*, **180**: 109–123.

Garbary, D.J. & Barkhouse, L.B. (1987). *Blidingia ramifera* (Bliding) stat. nov. (Chlorophyta): a new marine alga for eastern North America. *Nordic Journal of Botany*, **7**: 359–363.

Gayral, P. (1965). *Monostroma* Thuret, *Ulvaria* Rupr. emend. Gayral, *Ulvopsis* Gayral. (Chlorophycées, Ulotrichales): structure, reproduction, cycles, position systématique. *Revue générale de botanique*, **72**: 627–638.

Guiry, M.D. & Guiry, G.M. (2018). *AlgaeBase*. World-wide electronic publication. National University of Ireland, Galway. http://www.algaebase.org.

Hayden, H.S. & Waaland J.R. (2002). Phylogenetic systematics of the Ulvaceae (Ulvales, Ulvophyceae) using chloroplast and nuclear DNA sequences. *Journal of Phycology*, **38**: 1200–1212.

Hayden, H.S. & Waaland J.R. (2004). A molecular systematic study of *Ulva* (Ulvaceae, Ulvales) from the northeast Pacific. *Phycologia*, **43**: 364–382.

Hayden, H.S., Blomster, J., Maggs, C.A., Silva, P.C., Stanhope, M.J. & Waaland, J.R. (2003). Linnaeus was right all along: *Ulva* and *Enteromorpha* are not distinct genera. *European Journal of Phycology*, **38**: 277–294.

Heesch, S., Broom, J.E., Neill, K.F., Farr, T.J., Dalen, J.L. & Nelson, W.A. (2009). *Ulva, Umbraulva* and *Gemina*: genetic survey of New Zealand taxa reveals diversity and introduced species. *European Journal of Phycology*, **44**: 143–154.

Hillis, D.M. (1987). Molecular versus morphological approaches to systematics. *Annual Review of Ecology and Systematics*, **18**: 23–42.

Hiraoka, M., Ichihara, K., Zhu, W., Shimada, S., Oka, N., Cui, J., Tsubaki, S. & He, P. (2017). Examination of species delimitation of ambiguous DNA-based *Ulva* (Ulvophyceae, Chlorophyta) clades by culturing and hybridisation. *Phycologia*, **56**: 517–532.

Hoeksema, B.W. & Van Den Hoek, C. (1983). The taxonomy of *Ulva* (Chlorophyceae) from the coastal region of Roscoff (Brittany, France). *Botanica Marina*, **26**: 65.

Horta, P., Bernardes Batista, M., Cunha, R.L. & Castilho, R. (2018). Sea lettuce systematics: lumping or splitting? *In BioRxiv*. https://doi.org/10.1101/413450.

Hughey, J.R., Maggs, C.A., Mineur, F., Jarvis, C., Miller, K. A., Shabaka, S.H. & Gabrielson, P.W. (2019). Genetic analysis of the Linnaean *Ulva lactuca* (Ulvales, Chlorophyta) holotype and related type specimens reveals name misapplications, unexpected origins, and new synonymies. Journal of Phycology, doi: 10.1111/jpy.12860.

Kaminski, E. (1980). Über Funde von *Enteromorpha jugoslavica* Bliding in der Kieler Förde (west. Ostsee). *Botanica Marina*, **23**: 309–312.

Katoh, K., Misawa, K., Kuma, K.-I. & Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, **30**: 3059–3066.

Kirkendale, L., Saunders, G.W. & Winberg, P. (2013). A molecular survey of *Ulva* (Chlorophyta) in temperate Australia reveals enhanced levels of cosmopolitanism. *Journal of Phycology*, **49**: 69–81.

Koeman, R.P.T. & Van den Hoek, C. (1984). The taxonomy of *Ulva* (Chlorophyceae) in the Netherlands. *British Phycological Journal*, **16**: 9–53.

Koeman, R. & Van den Hoek, C. (1982a). The taxonomy of *Enteromorpha* Link, 1820, (Chlorophyceae) in the Netherlands I: the section *Enteromorpha*. *Archiv für Hydrobiologie*, **63**: 279–330.

Koeman, R. & Van den Hoek, C. (1982b). The taxonomy of *Enteromorpha* Link, 1820, (Chlorophyceae) in the Netherlands I. The section Proliferae. *Cryptogamie, Algologie*, **3**: 37–70.

Koeman, R. & Van den Hoek, C. (1984). The taxonomy of *Enteromorpha* Link, 1820, (Chlorophyceae) in the Netherlands III. The sections *Flexuosae* and *Clathratae* and an addition to the section *Proliferae*. *Cryptogamie, Algologie*, **5**: 21–61.

Kornmann, P. & Sahling, P.-H. (1977). Meeresalgen von Helgoland. *Helgoländer wissenschaftliche Meeresuntersuchungen*, **29**: 1–289.

Kornmann, P. & Sahling, P.-H. (1978). Die *Blidingia*-Arten von Helgoland (Ulvales, Chlorophyta). *Helgoländer wissenschaftliche Meeresuntersuchungen*, **31**: 391–413.

Kornmann, P. & Sahling, P.-H. (1983). Meeresalgen von Helgoland, Ergänzung. *Helgoländer wissenschaftliche Meeresuntersuchungen*, **36**: 1–65.

Kornmann, P. & Sahling, P.-H. (1994). Marine algae of Helgoland – 2nd Supplement. *Helgoländer Meeresuntersuchungen*, **48**: 365–406.

Kraft, L.G.K., Kraft, G.T. & Waller, R.F. (2010). Investigations into southern Australian *Ulva* (Ulvophyceae, Chlorophyta) taxonomy and molecular phylogeny indicate both cosmopolitanism and endemic cryptic species. *Journal of Phycology*, **46**: 1257–1277.

Kützing, F.T. (1843). *Phycologia generalis: oder, Anatomie, Physiologie und Systemkunde der Tange*. Brockhaus, Leipzig.

Lackschewitz, D., Reise, K., Buschbaum, C. & Karez, R. (2015). *Neobiota in deutschen Küstengewässern. Eingeschleppte und kryptogene Tier- und Pflanzenarten an der deutschen Nord- und Ostseeküste*. Landesamt für Landwirtschaft, Umwelt und ländliche Räume des Landes Schleswig-Holstein (LLUR).

Linnaeus, C. (1753). *Species Plantarum, Exhibentes Plantas Rite Cognitas Ad Genera Relatas: Cum Differentiis Specificis, Nominibus Trivialibus, Synonymis Selectis, Locis Natalibus, Secundum Systema Sexuale Digestas*. Trattner, Stockholm.

Maggs, C.A. & Kelly, J. (2007). *Percursaria*. In *The Green Seaweeds of Britain and Ireland* (Brodie, J., Maggs, C.A. & John, D., editors). British Phycological Society, London.

Maggs, C.A., Blomster, J. & Kelly, J. (2007a). *Umbraulva*. In *The Green Seaweeds of Britain and Ireland* (Brodie, J., Maggs, C.A. & John, D., editors). British Phycological Society, London.

Maggs, C.A., Blomster, J., Mineur, F. & Kelly, J. (2007b). *Ulva*. In *The Green Seaweeds of Britain and Ireland* (Brodie, J., Maggs, C.A. & John, D., editors). British Phycological Society, London.

Nylander, J.A.A. (2004). MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Pankow, H. (1990). *Ostsee-Algenflora*. Gustav Fischer Verlag, Jena.

Reed, R.H. & Russell, G. (1978). Salinity fluctuations and their influence on bottle brush morphogenesis in *Enteromorpha intestinalis* (L) Link. *British Phycological Journal*, **13**: 149–153.

Reinke, J. (1889). Notiz über die Vegetationsverhältnisse in der deutschen Bucht der Nordsee. *Berichte der Deutschen Botanischen Gesellschaft*, **7**: 367–369.

Rönnbäck, P., Kautsky, N., Pihl, L., Troell, M., Soerqvist, T. & Wennhage, H. (2007). Ecosystem goods and services from Swedish coastal habitats: identification, valuation, and implications of ecosystem shifts. *Ambio*, **36**: 534–544.

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, **61**: 539–542.

Rothmaler, W. (1984). *Exkursionsflora für die Gebiete der DDR und BRD Band 1: Niedere Pflanzen*. Volk und Wissen Volkseigener Verlag, Berlin.

Saunders, G.W. & Kucera, H. (2010). An evaluation of *rbc*L, *tuf*A, UPA, LSU and ITS as DNA barcode markers for the marine green macroalgae. *Cryptogamie, Algologie*, **31**: 487–528.

Schories, D., Selig, U. & Schubert, H. (2009). *Species and synonym list of the German marine macroalgae based on historical and recent records*. Meeresbiologische Beiträge, University of Rostock.

Shimada, S., Hiraoka, M., Nabata, S., Iima, M. & Masuda, M. (2003). Molecular phylogenetic analyses of the Japanese *Ulva* and *Enteromorpha* (Ulvales, Ulvophyceae), with special reference to the free-floating *Ulva*. *Phycological Research*, **51**: 99–108.

Smetacek, V. & Zingone, A. (2013). Green and golden seaweed tides on the rise. *Nature*, **504**: 84–88.

Spoerner, M., Wichard, T., Bachhuber, T., Stratmann, J. & Oertel, W. (2012). Growth and thallus morphogenesis of *Ulva mutabilis* (Chlorophyta) depends on a combination of two bacterial species excreting regulatory factor. *Journal of Phycology*, **48**: 1433–1447.

Staats, M., Cuenca, A., Richardson, J.E., Vrielink-van Ginkel, R., Petersen, G., Seberg, O. & Bakker, F.T. (2011). DNA damage in plant herbarium tissue. *PLoS ONE*, **6**: e28448.

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, **30**: 1312–1313.

Steinhagen, S., Karez, R. & Weinberger, F. (2018a). Molecular analysis of *Ulva compressa* (Chlorophyta, Ulvales) reveals its morphological plasticity, distribution and potential invasiveness on German North Sea and Baltic Sea coasts. *European Journal of Phycology*, **54**: 102–114.

Steinhagen, S., Karez, R. & Weinberger, F. (2018b). Surveying seaweeds from the Ulvales and Fucales in the world's most frequently used artificial waterway, the Kiel Canal. *Botanica Marina*, **62**: 51–61.

Tan, I.H., Blomster, J., Hansen, G., Leskinen, E., Maggs, C.A., Mann, D.G., Sluimam, H.J. & Stanhope, M.J. (1999). Molecular phylogenetic evidence for a reversible morphogenetic switch controlling the gross morphology of two common genera of green seaweeds, *Ulva* and *Enteromorpha*. *Molecular Biology and Evolution*, **16**: 1011–1018.

Vinogradova, K.L. (1969). K. sistematike poryadka Ulvales (Chlorophyta) s.l. A contribution to the taxonomy of the order Ulvales. *Botanicheskij Zhurnal SSSR*, **54**: 1347–1355.

Weinberger, F., Steinhagen, S., Afanasyev, D.F. & Karez, R. (2018). New records from the southern North Sea and first records from the Baltic Sea of *Kornmannia leptoderma*. *Botanica Marina*, **62**: 63–73.

Wichard, T. (2015). Exploring bacteria-induced growth and morphogenesis in the green macroalga order Ulvales (Chlorophyta). *Frontiers in Plant Science*, **6**.

Wolf, M.A., Sciuto, K., Andreoli, C. & Moro, I. (2012). *Ulva* (Chlorophyta, Ulvales) biodiversity in the North Adriatic Sea (Mediterranean, Italy): cryptic species and new introductions. *Journal of Phycology*, **48**: 1510–1521.

Womersley, H.B.S. (1984). *The Marine Benthic Flora of Southern Australia*. Government Printer, Adelaide.